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LEAF ARCHITECTURE, ORNAMENTATION AND ESTIMATION OF LAMINA AREA IN *MYRTUS COMMUNIS* L. (MYRTACEAE)

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ABSTRACT

Leaf architecture, ornamentation and estimation of lamina area have been described in a Mediterranean species, *Myrtus communis* grown under irrigation in Gulshan-e-Maymar, Karachi, Pakistan. Leaves opposite usually in 3's but same twig may also show superposed opposite leaves in 2's. Leaves simple, sub-sessile (petiole c 1mm) fleshy succulent, fine-textured, dark lustrous, green, stiff, and pinnately –veined and more shining dorsally. The main vein is paler in colour, impressed above and raised beneath (Ventral surface). Venation improminent except midrib. Dorsal surface is more shining than ventral surface. Lamina ovate-lanceolate. The apex angle (AA) averaged to 60.52 ± 1.17^{o} and base angle (BA) averaged to 71.64 ± 0.77^{o} i.e. BA was significantly larger than AA by a quantum of 11.12^{o} ($t = 10.22$, $p < 0.0001$). AA and BA were mostly acute, only rarely obtuse in case of few deformed leaves. Leaves are aromatic when bruised. Smell is refreshing but taste is bitter. Aspect ratio of leaf (LB / LL) was found to be 0.4238 ± 0.0065 varying by a quantum of 15.42% i.e. the leaf shape was fairly consistent in *M. communis*. Young stem and leaf pubescent - hairs simple, unicellular, non-glandular, solitary, conical or slightly wavy. The scent glands were present both dorsally and ventrally. Scent glands density was quite higher (966.63 ± 15.212 per cm^2) near dorsal epidermis than that near ventral epidermis (736.97 ± 16.563 per cm^2). Scent glands averaged to 95.30 ± 1.52 µm (N= 150) in diameter varying from 62.50 to 150.0 µm. Leaf Hypostomatous. Stomata anomocytic but staurocytic on petals. The stomatal density on mature leaf averaged to 500.74 ± 8.1 per mm^2. Stomatal size averaged to 20.36 ± 0.34 µm in length and 17.29 ± 0.27 µm in breadth. With around 10.8% variation, the magnitude of multiplication factor k averaged to 0.6337 ± 0.0623 (0.4545-0.8485) – quite reliable for determination of lamina area using linear dimensions of lamina length and breadth.

Key-words*: Myrtus communis,* leaf architecture and ornamentation, lamina area estimation.

INTRODUCTION

Myrtus communis L., native to Mediterranean region, belongs to Myrtaceae, a large family of 5500 species (Retamales *et al*., 2014). It bears 58 names in different languages of which myrtle is the most common names (gernet-ketzers-spice-ages.com/eng/index.htm).The plant is assigned to the goddess of Aphrodite (Venus). In Bible it is mentioned as a symbol for the blessings of God. It is considered as symbol of the Garden of Eden (quickbooker.org/kunden/wildherbs of crete.com/pages/portraits-of-our-essential-oil-from-wild-herbs-of-crete/ myrtle-myrtus communis.php?lang-DE). In Unani pharmacopeia, *M. communis* L. is known as Aas and its fruits are referred to Habb-ul-Aas (Sumbul *et al.,* 2011). There are remedies for a variety of ailments in *M. communis*. Its fruits and leaves are medicinal and have been used in many ailments. In past, its fruits were used as food ingredient due to their high Vitamin C content (Sumbul *et al.,* 2011). Rotondi *et al.* (2003) have studied the leaves of the species characteristic to the nature reserve "Arca di Noè" in a coastal area of North-Western Sardinia, Italy (40°36'N, 8°9'E;74 m above sea level) where *Myrtus communis* is one of the component species. The climate of the reserve is typical of mid-latitude Mediterranean islands. The vegetation of the area is distinguished in two main zones: a typical Mediterranean macchia and a coastal garigue, where stones and calcareous rocks surround small shrubs and herbaceous species. Most of the rainfall occurs in winter and autumn and summers are hot and dry (Chessa *et al.,* 1999; Delitala *et al.,* 2000) and temperatures are those of the subtropics moderated by maritime influence. *M. communis* is an evergreen sclerophyllous species (Yadav *et al.,* 2004) and it is said to be tolerant to drought (Gratini *et al*., 2013) in the Mediterranean climate. Aslam *et al.* (2010) have recorded *M. Communis* from Kashmir Himalaya. It had been introduced in the valley in lawns, parks and gardens for its sweet fragrance and ornamental value. Sara *et al.* (2012) collected it from Peshawar.

The studies pertaining to leaf architecture; ornamentation and estimation of lamina area have been undertaken in this paper on *M. communis* grown under irrigation in Gulshan-e-Maymar, Karachi, Pakistan where aridity is the basic characteristic of the physical environment. Koppen's (1918, 1936) classifications of world climate place this area under BWhw or hot desert climate. Trewartha (1954) puts the area in BW category, which is dry arid desert climate. According to this system Karachi is situated at the borderline of BS and BW types of climate (BS signifying dry semi-arid (steppe) climate. The bioclimate in accordance with Holdridge's (1947) system fall into the category, "Tropical desert bush formation". Rainfall is below 200mm per annum – mostly in summer. The Insolation in

summer is intense. The solar radiation is around 180-200 Kcal.cm^{-2}.Year $^{-1}$ causing glare and visibility reduction (Anna Mani *et al.*, 1965; Budyko, 1980). With an error of 4 - 5% the annual potential evapo-transpiration in the area amounts to 1750 mm (Zubenok, 1977). Solar global radiation (beam + diffused) as measured at Karachi varied from 3581 Kcal.m^{-2}.day^{-1} for December (15.04 MJ.m^{-2}.day^{-1}) to 5609 Kcal.m^{-2}.day^{-1} (23.56 MJ.m^{-2}.day^{-1}) for May. The diffused radiation is c. 20% of the global radiation (Ahmad *et al*., 1991).

MATERIALS AND METHODS

The twigs of Myrtle were sampled from a plant growing in Gulshan-e-Maymar, Karachi, in March 2015 and it was studied for leaf architecture, ornamentation and leaf area estimation. One hundred leaves of various sizes were randomly selected from the sampled twigs (Fig.1A) and the linear measurements from these leaves were recorded i.e. leaf length (LL) and leaf breadth (LB) at the broadest points on the margins. To measure the leaf area the outlines of the leaves were drawn on graph paper and the area was measured with all possible accuracy. The multiplication factor, k, was calculated by employing formula, k = leaf area $_{measured}$ / (LL x LB). And leaf area from linear dimensions was estimated as area $_{estimated}$ = k (LL x LB) (Lu *et al.*, 2012). For estimation of leaf area, average value of the k factor was employed.

Hickey (1973) and LAWG (1999) were followed for description of leaf architecture. Leaf epidermal impressions were made with clear nail polish (Wang *et al.*, 2006). Stomatal nomenclature suggested by Prabhakar (2004) being simple and based upon structure of stomata and not their ontogenetic pathways was adopted to ascertain stomatal types. This nomenclature does not recognize actinocytic and stephanocytic stomata and categorize them as anomocytic type. As a basic criterion, all the cells abutting the guard cells are considered distinct by Prabhakar (2004) from the other epidermal cells by virtue of their position (i.e. abutting nature to the guard cells) hence he prefers to call them subsidiaries. Scent glands and stomatal density and their size were determined microscopically in µm with calibrated micrometer. The parameters of leaf histology were measured in a transverse section of an average-sized leaf (2.5 x 1.0cm). The data was analyzed statistically (Zar, 2010). The skewness and kurtosis and their errors were calculated following Shaukat and Khan (1979). The openings of the scent glands on dorsal and ventral surfaces of leaf were counted microscopically under magnification of 10 x 10 X. Each frame or field of vision under this magnification covered an area of 1.76625 mm^2. The size (diameter) of the gland was measured under 45 x 15 X magnification.

Fig. 1. Habit of *Myrtus communis* L. (A). Whorled nature of leaves (B) and opposite superposed leaves in pairs (C).

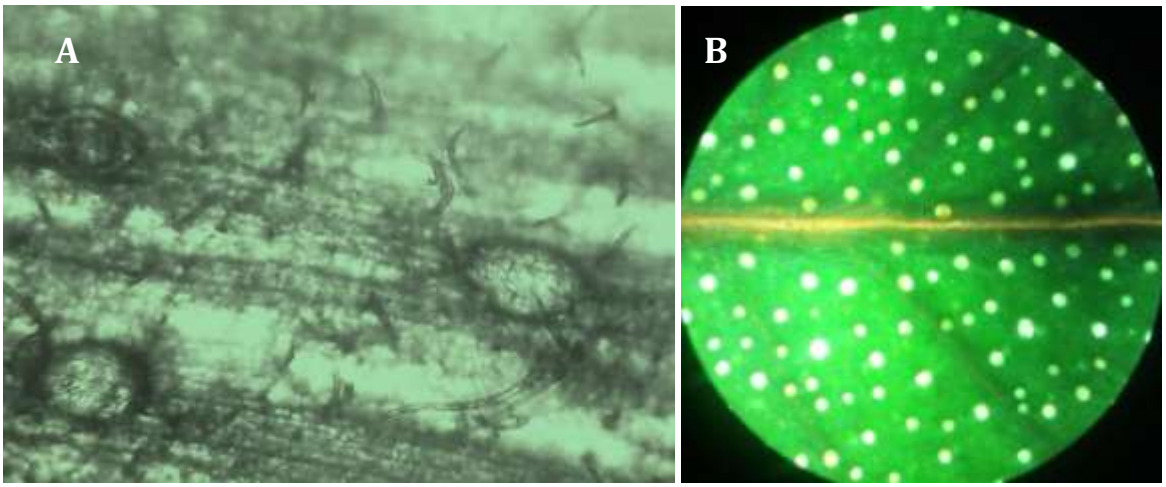


Fig. 2. Stem surface showing areas of the scent cavities and trichomes (A) and scent glands seen on dorsal surface of leaf (B) – yellowish brown central streak is the midrib. Lateral veins as silhouettes also visible (10 x 5 X).

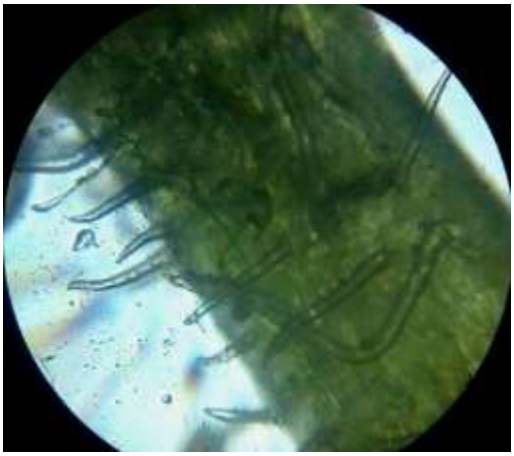


Fig. 3. Enlarged view of trichomes on stem surface.

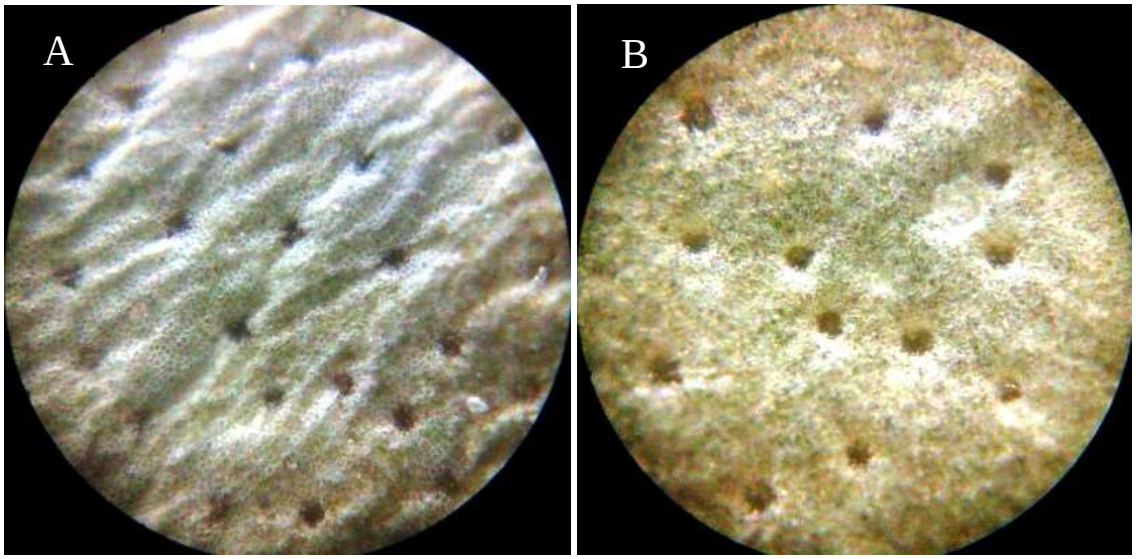


Fig. 4. Scent glands distributed on leaf - A, dorsal and B, Ventral surface. 10 x 10 X. Under microscope scent glands appear as round depressions on dorsal and ventral surfaces. These areas correspond to the internal structures, the scent glands.

RESULTS AND DISCUSSION

M. communis is a strongly scented upright shrub with beautiful white blossoms. It was copiously flowering and fruiting in March in Karachi. Aslam *et al.* (2010), however, reported it to flower from July to September in Kashmir Himalaya. The leaves-bearing stem is green when very young but turn to red-brown in colour as it matures (Fig. 1A and B). The branching is di- to trichotomous. The stem is pubescent with unicellular hairs and also bears scent glands (Fig. 2 A and 3).

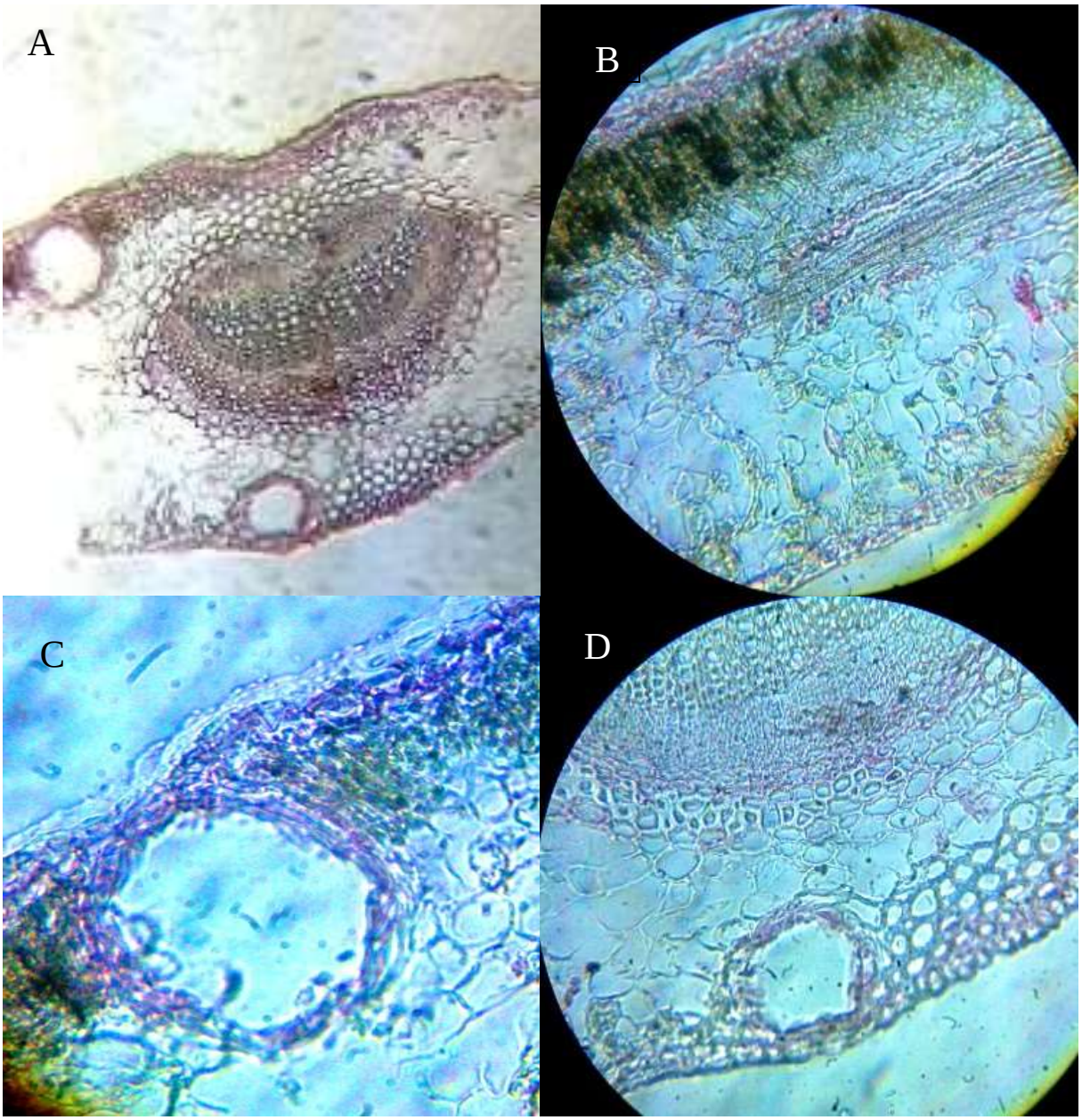


Fig. 5. Histology of leaf. A, T.S. midrib region showing vascular bundle with sheath and scent glands with upper and lower epidermises; B, T.S of leaf lateral lamina showing upper epidermis followed by palisade and thicker region of spongy tissue; C, scent gland just below epidermis on dorsal surface in palisade region - Cuticular layer above epidermis is clearly visible as white band; Lining of scent gland cavity with secretory cells derived with periclinal division is clearly visible. D, Scent gland just beneath lower epidermis. Above the scent gland is the midrib vascular bundle.

Table 1. Location and dispersion of leaf architectural and lamina area parameters of *Myrtus communis* leaves.

Statistical Parameters	LL (cm)	LB (cm)	K	LAM (cm^2)	Aspect ratio*	Apex angle (^{o})	Base angle (^{o})	LAK (cm^2)	LAMR (cm^2)	LAPOW (cm^2)
N	100	100	100	100	100	100	100	100	100	100
Mean	2.693	1.1243	0.633698	1.9471	0.42377	60.52	71.64	1.9677	1.94695	1.94092
SE	0.0526	0.02082	0.062285	0.06268	0.006533	1.171	0.770	0.06809	0.06013	0.06294
Median	2.80	1.10	0.631098	1.940	0.41404	58.0	70.0	1.8954	1.8923	1.8809
CV (%)	19.52	18.52	10.93	32.19	15.42	2.25	10.75	34.61	30.89	32.43
Skewness	-0.321	0.280	0.162	0.231	0.855	1.360	0.891	0.571	0.076	0.505
Kurtosis	-0.098	0.169	0.843	0.190	1.20	2.446	2.429	0.228	0.465	0.208
Minimum	1.20	0.55	0.4545	0.45	0.300	40	55.0	0.4182	0.1478	0.4575
Maximum	3.80	1.60	0.8485	3.58	0.6471	105	100.0	3.6121	3.1820	3.4384
KS-z	1.009	1.082	0.833	0.806	0.864	0.477	1.114	1.128	1.040	1.095
P	0.261	0.193	0.491	0.534	0.444	0.025	0.167	0.157	0.230	0.181

LL, Lamina length (cm); LB, lamina breadth (cm); LL x LB (Lamina length x lamina breadth multiplicative parameter; k, multiplication factor; LAM, Leaf area measured; Aspect ratio, LB/LL; LAK, k-based area estimated; LAMR, leaf area estimated on the basis of multiple regression with LL and LB; LAPOW, leaf area based on power equation (with LL x LB as independent variable.
G1, skewness; g2, kurtosis; Sg1; SE of skewness = 0.241 and Sg2; SE of kurtosis = 0.478

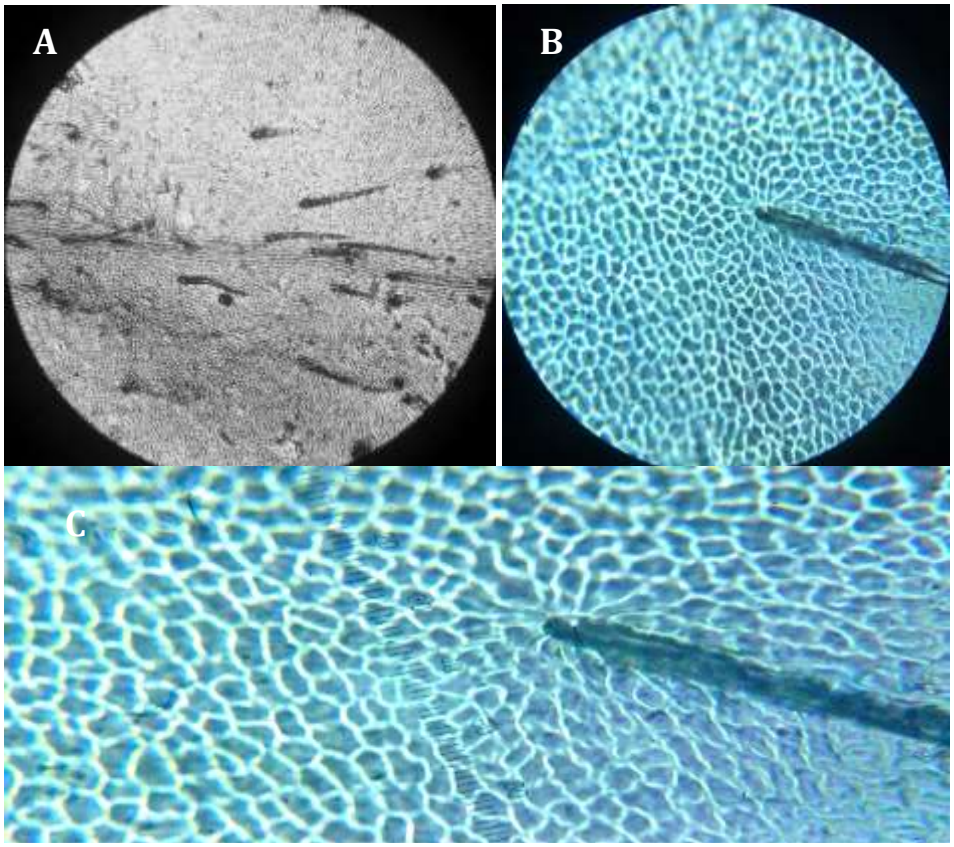
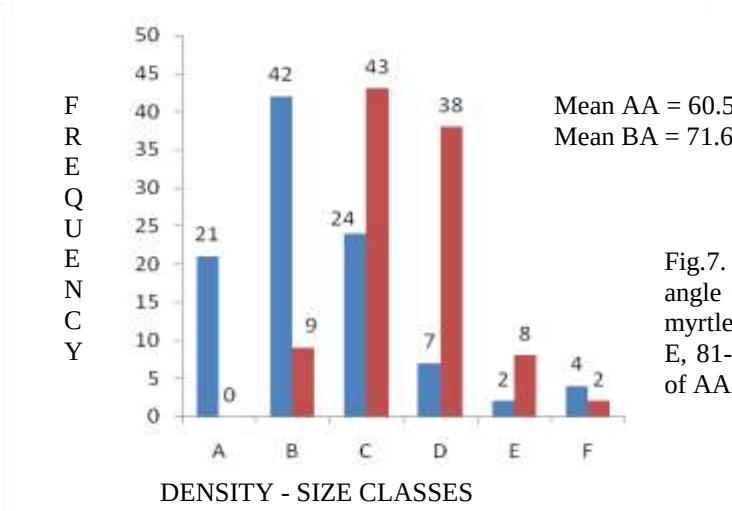


Fig. 6. Dorsal surface of leaf showing trichome - on and near the midrib (A, 10 x 10 X); on the lamina surface (B and C, 45 x 10 X and 45 x 15 X, respectively). There appear no stomata on the dorsal surface (C). Note the epidermal cells are polyhedral and they are arranged in a ring around the trichome base.

Leaf architecture

Leaves opposite usually in 3's but same twig may also show superposed opposite leaves in 2's (Fig. 1C). Leaves are simple, sub-sessile (petiole c 1mm) fleshy succulent, fine-textured, dark lustrous, green, stiff, and pinnately –veined. The main vein is paler in colour, impressed above and raised beneath (Ventral surface). Venation improminent except midrib. Dorsal surface is more shining than ventral surface. Lower pair of leaves of a branch is smaller in size than subsequent upper pairs. Lamina ovate-lanceolate. The apex angle (AA) averaged to 60.52 ± 1.17^o and base angle (BA) averaged to 71.64 ± 0.77^o (Table 1) i.e. BA was significantly larger than AA by a quantum of 11.12^o (t = 10.22, p< 0.0001). AA and BA were mostly acute, only few obtuse (4 and 2%, respectively) in case of few deformed leaves (Table 1, Fig. 7). Variation in BA was comparatively larger (10.75%) than in AA (2.25%).

Both apex and base extension lengths (La and Lb) are zero. The leaf is more shining dorsally than ventrally. It is aromatic when bruised. Smell is refreshing but taste is bitter. Aspect ratio of leaf (LB / LL) was found to be 0.4238 ± 0.0065 varying by a quantum of 15.42% i.e. the leaf shape was fairly consistent in *M. communis*.



Mean AA = 60.52 ± 1.17^o (Blue bars)
Mean BA = 71.64 ± 0.77^o (Red bars)

Fig.7. Per cent frequency of size classes of apex angle (AA) and base angle (BA) of 100 leaves of myrtle. A, < 50^o; B, 51-60^o; C, 61-70^o; D, 71-80^o; E, 81-90^o; F, > 90^o (maximally up to 105^o in case of AA and 100^o in case of BA.

Leaf histology

The leaf histology of a leaf (2.5 x 1.0cm in size) is depicted in Fig. 5 and histological parameters are presented in Table 2. The leaf is dorsiventral. Epidermis is single layered, 16.4 to 19.7 µm in thickness with a layer of cuticle and wax, around 3 to 4 µm thick. The leaf thickness at midrib was 525 µm, 1.62 times of the lamina thickness. Adjacent to the upper epidermis was palisade followed by spongy tissue which was 4-times in thickness as compared to the palisade. Scent glands were distributed in palisade and spongy tissue areas, sometimes below the vascular bundle in midrib zone. Our measurements of the histological parameters for myrtle leaf are almost comparable to the histological parameters of the myrtle leaf reported by Rotondi *et al.* (2003) from "Arca di Noè" of North –Western Sardinia, Italy. Myrtle by its histology appeared to be xerophytic plant with cuticular lining covering both epidermises to reduce transpiration and the presence of trichomes. This feature is common in almost all drought resisting plants of "Arca di Noè" studied by Rotondi *et al*. (2003).

Leaf ornamentation

a) Trichomes

Leaf is pubescent with unicellular non-glandular solitary hairs scattered sparingly over the leaf surface (dorsal as well as ventral) but more in number near or on midrib (Fig. 6A, 11). Since trichomes are delicately thin and wither soon, the number of trichomes decline with age. The mature leaves thus have trichomes but sparingly. The base of the trichome is surrounded by several epidermal cells arranged around (Fig.6C). When trichome is broken from base, the scar is formed with more or less circular hollowing (Fig. 13E and F). The hairs are simple, unicellular, non-glandular, solitary, conical, curved and slightly wavy.

The adaxial surface of leaves of *Ugni molinae*, another ornamental plant of family Myrtaceae is reported to be glabrous while abaxially it has some scattered hairs. Like myrtle, the hairs in *Ugni* are also simple, unicellular, non-glandular, solitary, conical and slightly wavy. Trichomes are abundant on midrib (Retamales *et al*., 2014).

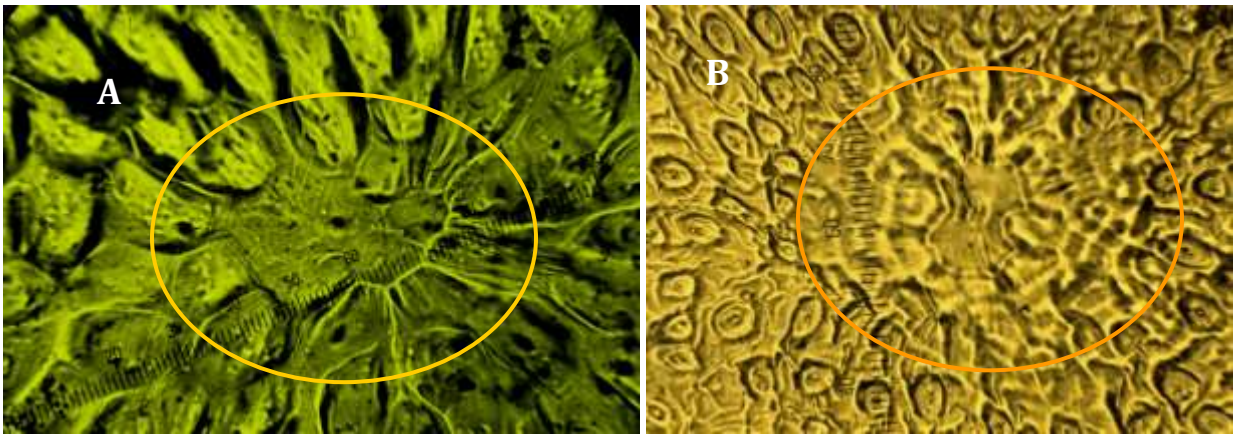


Fig. 8. Hollowing of the cells of the dorsal foliar epidermis just above the scent gland (A) and another scent gland area on the ventral surface of leaf (B) – several stomata are also visible..

Table 2. Parameters of leaf histology – measurements based on transverse section* of a leaf (2.5 x 1.0cm).

S. No.	Leaf Tissue	Measurement (µm)	Data from Rotondi *et al.* (2003)**
1	Upper cuticle + wax thickness	3-4	2
2	Lower cuticle + wax thickness	3-4	2
3	Upper epidermis thickness	16.4-19.7	18
4	Lower epidermis thickness	16.4-19.7	14
5	Leaf thickness at midrib	525	-
6	Lamina thickness	325	291
7	Palisade thickness	62.5-75.0	51
8	Spongy tissue thickness	250-312.5	204
9	Main V.B. breadth	437.5	-
10	Main V.B. thickness	250.0	-

*, Leaf transverse section from the region of leaf breadth = 6062.5µm; **, Mediterranean climate.

b) Scent glands

Under microscope scent glands may be identified as round structure sinking into surfaces of leaf, dorsal and ventral. These areas correspond to the internal glands. The scent glands are present on both surfaces of leaf. The scent glands on dorsal and ventral surfaces of leaf are shown in Fig. 4. The cellular view of epidermis corresponding to internal glands are presented in Fig. 8. Scent glands are formed in palisade or spongy tissue region adjoining epidermis (Fig. 5) although they may occur in the midrib region as well (Fig. 5). The scent glands had no aperture or any opening on the epidermis which is in agreement with Kalachanis and Psaras, 2005). The presence of scent gland is a common feature in family Myrtaceae (Metcalfe and Chalk, 1979). The secretory cavities of scent glands, according to Kalachanis and Psaras (2005), are continuously formed during leaf development but in mature leaf the rhythm of their appearance shows steep decrease. Each cavity is developed from a single epidermal cell which undergoes periclinal division followed by anticlinal and several oblique cell divisions. The lumen of the secretory cavity is initiated by cell wall separation i.e. schizogeneously. Ciccarelli *et al.* (2003; 2008) have also described the development of secretory cavity in *M. communis*. The ontogeny of these cavities follows a schizo-lysigeneous development (indeed both schizogenous and combination of schizogenous and lysigenous development). Their origin is suggested from the protoderm with participation of ground meristem (Arruda and Fontenelelle, 1994; Fahn, 1979). The secretory cells line up the cavity, where the secreted material is collected. The secretory cavities are covered by modified epidermal cells (cf. Fig. 8) which do not seem to form any special aperture. The essential oil seems to be the discharge after mechanical treatment to the leaf (Kalachanis and Psaras, 2005). The composition of essential oil varies with different locations (Gernot-katzers-spice-pages.com/engl/index.html).

Scent glands density was quite higher (966.63 ± 15.212 per cm^2) on the dorsal surface (Fig. 9) than that on the ventral surface (736.97 ± 16.563 per cm^2) (Fig. 10). The density of foliar scent glands on dorsal surface varied from 509 to 1246 per cm^2; CV=13.63%). The density size class of 600-900 glands per cm^2 was the largest class

occupying 77.91%. The density of scent glands on the ventral surface varied from 339.0 to 962.5 per cm^2 (Fig. 10). As seen under 10 x 10X magnification scent gland averaged to 95.30 ± 1.52 µm (N= 150) in diameter varying from 62.50 to 150.0 µm (CV= 19.58%). Correspondingly, the scent gland area averaged to 0.0074 ± 0.000238 mm^2 (varying from 0.0031 to 0.0177 mm^2).

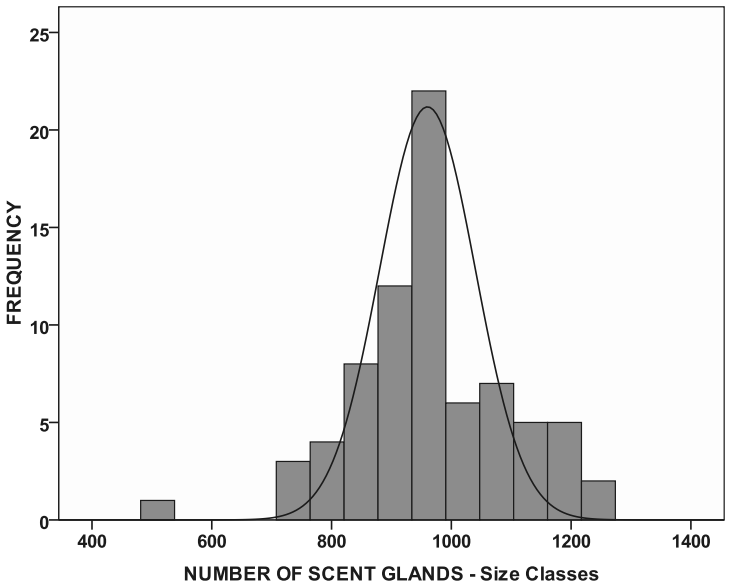


Fig. 9. Frequency distribution of scent glands per cm^2 on the dorsal surface of mature leaf.

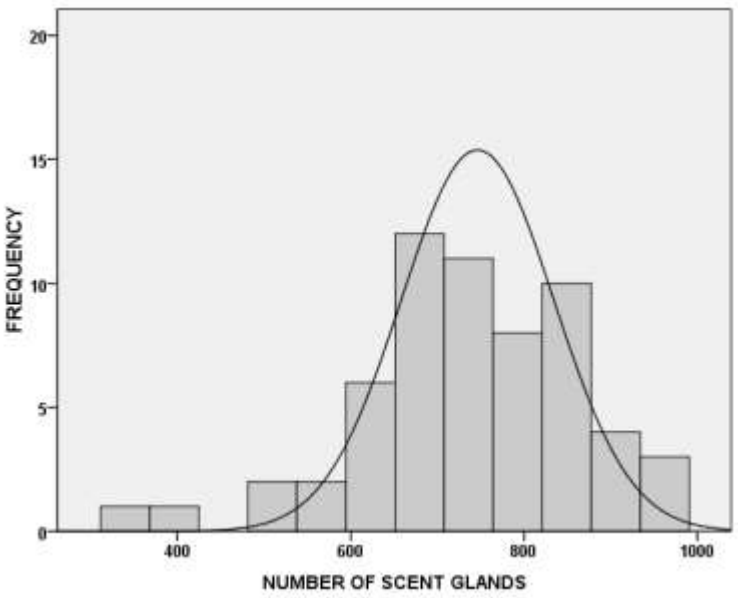


Fig. 10. Frequency distribution of scent glands per cm^2 on the ventral surface of mature leaf.

Stomata

Although thick leaves tend to be amphistomatous, the leaves of *M. communis* were hypostomatic – stomata on the ventral foliar surface only. Hypostomatous leaves, as studied by Parkhurst, 1978) with herbarium species of several families, occurred least often in xeric habitats, more often in mesic ones and again often in hydric habitats. Hypostomatous leaves were more prominent over hyperstomatous ones. The stomata in myrtle are of anomocytic type (Fig. 11A). The stomata are generally small in size but few of them are quite larger in size (Fig, 11A and 13).

On petal's outer surface, however, staurocytic type of stomata surrounded by four subsidiaries was observed (Fig.11 B). On ventral surface, several contiguous stomata were observed (Fig. 13 A, B, C and D).

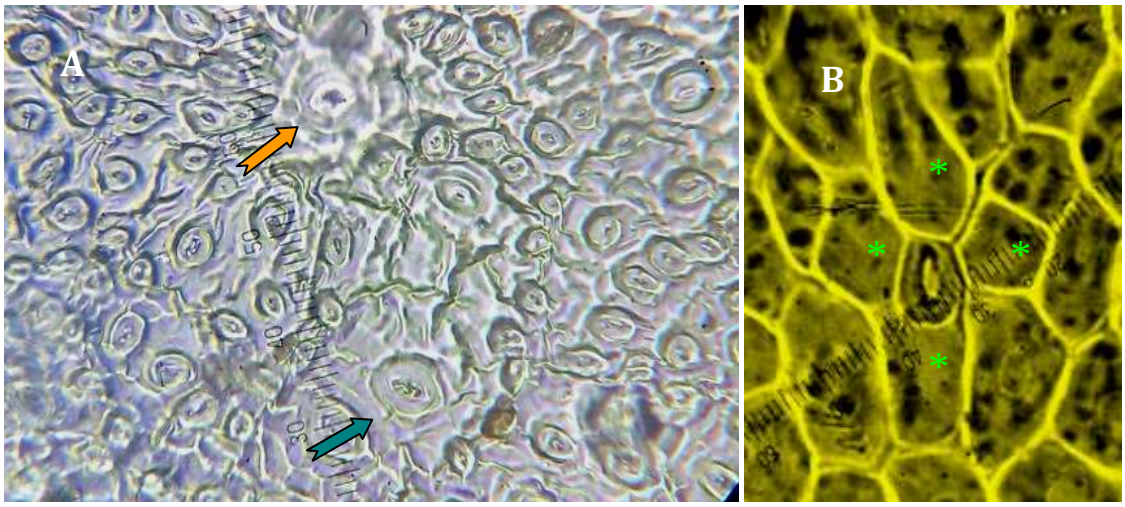
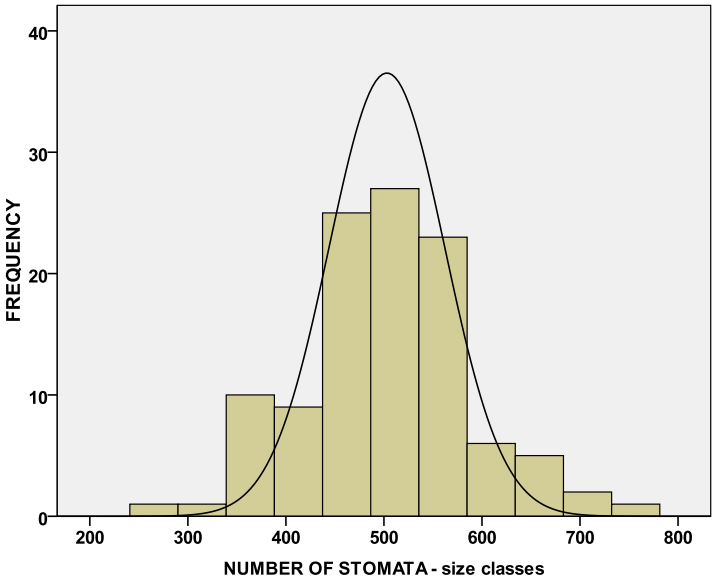


Fig. 11. (A) Stomata (anomocytic) on ventral surface of leaf (Green arrow). Indicated by an orange arrow is the scar due to a broken trichome. (B) A stoma on the outer surface of the petal.



N = 110
Mean = 500.74
SE = 8.0975
Median = 506.19
CV (%) = 16.96
G1 = 0.117
Sg1 = 0.230
G2 = 0.477
Sg2 = 0.457
Minimum = 265.4
Maximum = 747.0
KS-z = 0.927
P < 0.357

Fig. 12. Frequency distribution of stomatal density per mm^2 on ventral surface of mature leaf.

Table 3. Stomatal size (stoma + guard cells) of myrtle leaf ventral surface.

Statistical parameter	Stomatal length (µm)	Stomatal breadth (µm)	Length / breadth ratio
N	125	125	125
Mean	20.362	17.292	1.198
SE	0.33592	0.26644	0.0155
Median	19.680	16.40	1.20
CV (%)	18.43	17.21	14.44
Minimum	13.12	9.084	0.86
Maximum	29.52	26.24	1.67

Table 4. Linear and power regression models of a multiplicative parameter (LL x LB) with LAM.

	Linear Regression of LAM with multiplicative parameter of LL x LB
LINEAR Model	LAM = 0.22581 + 0.554364 (LL x LB) ± 0.19580 t = 3.76 t = 30.27 P < 0.00003 p < 0.00001 R^2 = 0.903; Adj. R^2 = 0.902; F = 916.454 (p < 0.000001)
	Power law relation of LAM with multiplicative parameter LL x LB
LAPOW Model	LAM = 0.674905. LL x LB $^{0.935498}$ ± 0.10834 t = 30.314 t = 32.11 p < 0.00001 p < 0.00001 R^2 = 0.913, Adj. R^2 = 0.912; F = 1030.95 (p <0.00001)

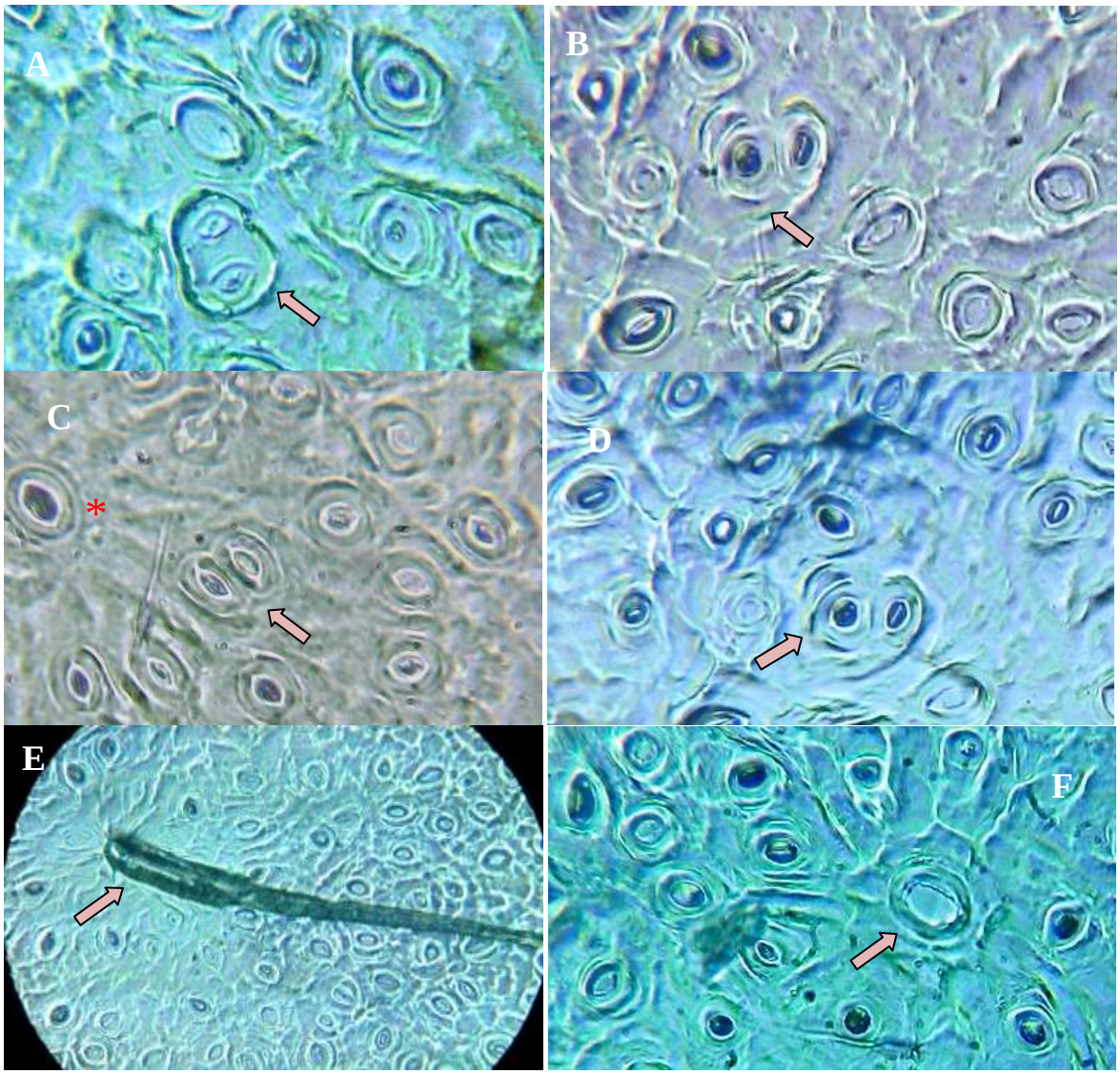
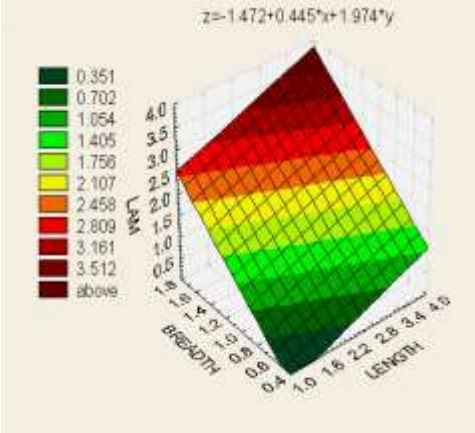


Fig. 13. Contiguous Stomata, without subsidiary cell (s) between them, on the ventral surface of leaves of *M. communis* (A, B, C, and D). A trichome on ventral surface (E) and a scar of the broken trichome (F). Marked with an asterisk (C) is the larger stoma.



LAM = -1.472 + 0.445 LL + 1.974 LB ± 0.17866
t = -14.19 t = 9.110 t = 16.01
p < 0.0001 p < 0.00001 p < 0.0001
R = 0.959, R^2 = 0.920. Adj R^2 = 0.919,
F = 560.74

	LL	LB
Zero order correlation:	0.843	0.923
Partial correlation:	0.679	0.852

Fig. 14. Surface plot of lamina length (cm) and breadth (cm) with Lamina area measured, cm^2) – in a multiple linear regression model (LAMR).

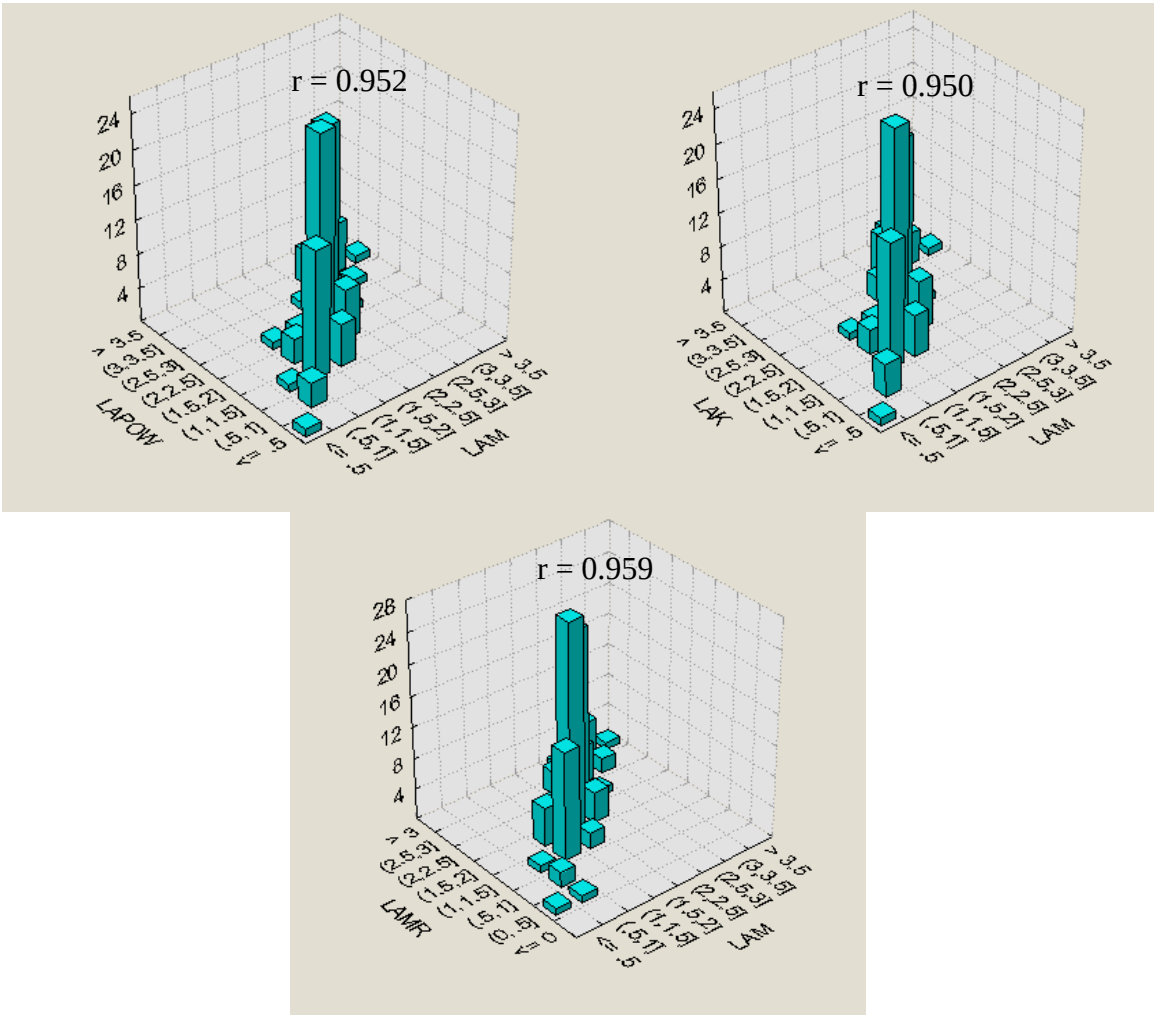


Fig. 15. Bivariate plots between leaf area measured (LAM, x-axis) and estimated leaf areas (Y-axis) such as LAPOW, LAK and LAMR.

Stomatal density and stomatal size

The stomatal density on the ventral surface of mature leaf was observed to average 500.74 ± 8.1per mm^2 varying around 17% and distributed normally (Fig. 12). Stomata were denser on the younger small- sized leaves than on the mature leaves. The lower stomatal density on mature leaf surface may probably be attributed to the foliar expansion of developing leaves with age. Stomatal density in *M. communis* has been quantified to be 456 per mm^2 by Christodoulakis and Mitrakos (2013), 394.7 ± 15.2 per mm^2 by Yadav *et al.* (2004) and 342 per mm^2 (Rotondi *et al.*, 2003) in Mediterranean samples. Stomatal density in the in-hand sample of myrtle (cultivated in Karachi, Pakistan) was comparable to the magnitude of stomatal density given by Christodoulakis and Mitrakos (2013). High stomatal density appears in agreement with typical xeromorphism. Stomatal size averaged to 20.36 ± 0.34 µm (CV = 18.43%) in length and 17.29 ± 0.27 µm in breadth (CV = 17.21%) (Table 3). Larger stomata are fewer in number.

Leaf area estimation

The lamina area related parameters are presented in Table 1. The leaf length averaged to 2.693 ± 0.0526 cm (1.20-3.80 cm) and tended to be normally distributed. Leaf breadth averaged to 1.124 ± 0.0623 cm (0.55 to 1.08 cm) and also distributed normally. These statistics were more or less comparable to those reported for leaf length (3.2 ± 0.3 cm) and breadth (1.2 ± 0.2 cm) in a myrtle sample from Malakasa, 37 km North of Athens, Greece (Christodoulakis and Mitrakos, 2013). With variation of 15.42% the measured area of leaf (LAM) averaged to 1.95 ± 0.6227 cm^2 (0.45 to 3.58 cm^2). Christodoulakis and Mitrakos (2013), without mentioning the variation, reported leaf size in *M. communis* from Greece to be 2.5 cm^2.

With around 10.8% variation, the magnitude of multiplication factor k averaged to 0.6337 ± 0.0623 (0.4545-0.8485) and distributed in normal fashion. Linear simple and multiple correlation and regression models obtained by regression of leaf area with their respective lengths or breadths separately or in combination or as power law predictive equations yielded significant results (Table 4 and Fig. 14). It was apparent that leaf breadth (LB) was somewhat better related to leaf area than leaf length (LL). The comparison of the models on the basis of r, r^2, and F ratio values suggested that the power law equation was relatively better to define leaf area on the basis of LL X LB – explanatory value of the equation being 91.3% (Table 4).

The leaf area in present studies, was also determined mathematically by employing average multiplication ratio or factor (k = 0.6337). The location and dispersion parameters of measured and estimated leaf areas (LAM, LAMR, LAPOW and LAK) are presented in Table 1. All these variables distributed normally. The measured and estimated average areas were not found to be significantly different from each other ($t_{LAM\ vs\ LAMR}$ = 0.087, NS; $t_{LAM\ vs\ LAK}$ = 0.56, NS and $t_{LAM\ vs\ LAPOW}$ = 0.071, NS. It is also evident from the bivariate distribution of these parameters portrayed in Fig. 15 and more or less similar intensity of correlation amongst them.

Above-given results on leaf area estimation of *M. communis* were highly significant and could be useful in experimental agronomic studies with this taxon. Since Huxley (1924) who was the first to undertake such studies, many workers have undertaken leaf area estimation allometrically as well as mathematically and have obtained useful results with many plant species e.g., *Fragaria* spp. (Demirsoy *et al.* (2005); *Xanthosoma* spp. (Goenaga and Chew (1991); *Arachis hypogaea* (Kathirvelan and Kalaiselvan, 2007); hazel nut (Cristofori *et al.* (2007); millet (Persaud *et al.* (1993); *Prunus avium* (Citadani and Peri, 2006); in 15 fruit spp. (Uzun and Celik, 1999); sunflower (Bange *et al.* (2000), grapevine (Williams, and Martinson (2003), cotton (Akram-Ghaderi and Sultani, 2007), *Nicotiana plumbaginifolia (*Khan, 2008*), improved genotypes of Coffea arabica* and *C. canephora* (Brinate *et al.*, 2015), *Concorde grape (*Elasner and Jubb, 1988*), Ficus religiosa* (Khan, 2009), *Ricinus communis* (Jain and Misra, 1966), *Jatropha curcas* (Ahmed and Khan, 2011), *Vicia faba* (Erdoğan, (2012), *Simmondsia chinensis* (Khan *et al.*, 2015a), *Capparis cartilaginea* (Khan *et al.*, 2015d), *Medicago sativa* (Khan *et al.*, 2016), in grasses (Kemp, 1960), etc. It is known that the environmental interactions may influence any such model in plants (Robbins and Pharr, 1987). However, in view of the simplicity, convenience and the accuracy of estimation, using mean k coefficient (k = 0.6337) while measuring length and breadth of the leaves may be recommended for the estimation of leaf area in *M. communis* cultivated under environment conditions of Karachi.

REFERENCES

Ahmad, F., S.M. Aqil Burney and S.A. Hussain (1991). Monthly average daily global and diffuse solar radiation and its correlation with hours of bright sunshine of Karachi, Pakistan. *Renewl. Energy* 1: 115-118.

Ahmed, N. and D. Khan (2011). Leaf area estimation in *Jatropha curcas* L. *Int. J. Biol. Biotech.*, 8(3): 301-307.

Akram-Ghaderi and F.A. Sultani (2007). Leaf area relationships to plant vegetative characteristics in cotton (*Gossypium hirsutum* L.) grown in temperate sub-humid environment. *Intern. J. Pl. Production*, 1(1): 63–71.

Anna Mani, O. Chacko, V. Krishnamurthy, and V. Desikan (1965). Radiation balance of Indian Ocean-Proc.

International Indian Ocean expedition, Bombay. pp. 165 - 177.

Arruda, R.C.O. and D.B. Fontenelle (1994). Anatomia foliar de Psidium Cattely anum. *Res. Bras. Der. Bot.* 17: 25-35.

Aslam, S., K.A., Gannair, A.Q. John, and G.H. Dar (2010). Family Myrtaceae in Kashmir: *Myrtus communis* L. A new record for the shrub world of Kashmir Himalaya. *Academia Arena* 2(5):42-43.

Bange, M.P. G.L. Hammer, S.P. Miloroy and K.G. Rickert (2000). Improving estimates of individual leaf area of sunflower. *Agronomy*, 92: 761–765.

Brinate, S.V.B., N.N. Rodrígues, L. Martin, D.T.V. Colodetti, M.A. Tomaz and J.F.T.D. Amaral (2015). Applicability of the method of linear dimensions to estimate leaf area in improved genotypes of *Coffea arabica* and *Coffea canephora. Am. J. Pl. Sci*., 6: 651-658.

Budyko, M. (1980). *Global Ecology*. Progress Publ. Moscow.

Chessa, P.A., D. Cesari and A. Delitala (1999). Regimes of precipitation and temperature in Sardinia (Italy) and the related synoptic situations. Theoretical and Applied Climatology 63: 195- 222. (Seen in Rotondi *et al.*, 2003).

Christodoulakis, N.S. and K.A. Mitrakos (2013). Structural analysis of sclerophylly in eleven evergreen phanerophytes in Greece. In: J.D. Tenhuen, Catarino, F.M., Lange, O.L. and Ochel, W.C. (Eds.).*Plant Response to Stress: Functional Analysis in Mediterranean Ecosystem*. Springer Sci. & Business Media, 2013. 668 Pp.

Ciccarelli, D., F. Garbari and A. M. Pagni (2008). The flower of *Myrtus communis* (Myrtaceae): secretory structures, unicellular papillae and their ecological role. *Flora-Morphology, Distribution, Functional Ecology of Plants* 203:85-93. (DOI: 10.1016/J.flora.2007.01002).

Ciccarelli, D., A.M. Pagni, A.M. and A.C. Andreucci (2003). Ontogeny of secretory cavities in vegetative parts of *Myrtus communis* L. (Myrtaceae): An example of schizogenous development. *Israel J. Pl. Sci.* 51: 193-198. (DOI: 10.1560/12F4-M3YH-WD2D-NF3B).

Citadiani, E.D. and P. L. Peri (2006). Estimation of leaf area in sweet cherry using a non-destructive method. *RIA,* 35 (1): 143–150.

Cristofori, V., Y. Rouphael, E. Mendoza-de Gyves and C. Bignami (2007). A simple model for estimating leaf area of hazel nut from linear measurements. *Sci. Hort*., 113(2): 221–225.

Delitala, A.M., D. Cesari, P.A. Chessa and N.M. Ward (2000). Precipitation regimes over Sardinia (Italy) during the 1946-1993 and associated large-scale climatic variations. International Journal of Climatology 20: 519-542. (Seen in Rotondi *et al.*, 2003).

Demirsoy, H., L. Demirsoy and A. Öztürk (2005). Improved model for the non-destructive estimation of strawberry leaf area. *Fruits,* 60: 69-73.

Elasner, E.A. and G.L. Jubb (1988). Leaf area estimation of Concord grape leaves from simple linear measurements. *Am. J. Enol. Vitic*. 39(1): 95 – 97.

Erdoğan, N.C. (2012). A leaf area estimation for faba bean (*Vicia faba* L.) grown in the Mediterranean type of climate. *Süleyman Demirel Üniversitesi Ziraat Fakültesi Dergisi*, 7(1): 58-63.

Fahn, A. (1979). *Secretory Tissues in Plants*. Academy Press, London.

Goenaga, R. and V. Chew (1991). Estimation of leaf area in three tanier (*Xanthosoma* spp.) cultivars grown in Puerto Rico. *J. Agric. Univ. Puerto Rico*, 75 (3): 313–315.

Gratini, L., R. Catoni, and L. Varone (2013). Morphological, anatomical and physiological leaf traits of *Q. ilex, P. latifolia, P. lentischus* and *M. communis* and their response to Mediterranean climate stress factors. *Bot. Studies* 54: 35(http://www.as.botanicalstudies.com/content/54/1/35)

Hickey, L.J. (1973). Classification of the architecture of dicotyledonous leaves. *Am. J. Bot.*, 60(1): 17-33.

Holdridge, L.R. (1947). Determination of World Plant Formations from simple Climatic Data. *Science* (NY) 105: 367 - 368.

Huxley, J.S. (1924). Constant differential growth ratios and their significance. *Nature* (London) 114: 895.

Jain, T.C. and D.K. Misra (1966). Leaf area estimation by linear measurements in *Ricinus communis*. *Nature,* 212: 741 – 742.

Kalachanis, D .and G. K. Psaras (2005). Structure and development of the secretory cavities of *Myrtus communis* leaves. *Biol. Plantarum* 49 (1): 105-110.

Kathirvelan, P. and P. Kalaiselvan (2007). Ground nut (*Arachis hypogaea* L.) leaf area estimation using allometric Model. *Res. J. Agric. & Biol. Sciences,* 3(1): 59 -61.

Kemp, C.D. (1960). Methods of estimating the leaf area of grasses from linear measurements. *Ann. Bot*., 24: 491 – 499.

Khan, D, M.J. Zaki, and S.M. Abbas (2015a). Leaf area estimation in Jojoba (*Simmondsia chinensis* (Link.) C.E. Schneider seedlings. *Int. J. Biol. Biotech., 12(4):* 667-674.

Khan, D. (2008). Plant-size data and estimation of some vital leaf characteristics in naturally growing *Nicotiana plumbaginifolia* Viv. (Solanaceae) in Karachi. *Int. J. Biol. Biotech*. (1 – 2): 111-123.

Khan, D. (2009). Estimation of some leaf traits in variously-aged cordate-acuminate leaves of *Ficus religiosa* L. *Int. J. Biol. Biotech*., 6(1-2): 51-60.

Khan, D., Z. Baig and M. Ahmed (2016). Leaf architecture, ornamentation and leaf area estimation in exotic *Medicago sativa* L. – grown in Pakistan. *Int. J. Biol. Biotech*. 13(3): 359-375.

Khan, D., S.S. Shaukat, S.S. and S.V. Ali (2015b). Leaf architecture and estimation of lamina area in *Capparis cartilaginea* Decne. *Int. J. Biol. Res*., 3(1): 19-29.

Köppen, W. (1918). Klassification der climate nach temperaturen Niederschlag und Jarhslaug, *Petermans mitt*. 64: 193.

Köppen, W. (1936). *Das Geographische system der Klimate Handbook*. der Klimatogie, Berlin.

LAWG (Leaf Architecture Working Group). (1999). *Manual of Leaf Architecture: Morphological description and Categorization of Dicotyledonous and Net-Veined Monocotyledonous Angiosperms.* Smithsonian Institution, USA. Pp. 65.

Lu, H., W. Jiang, W.M. Ghiassi, S. Lee, and M. Nitin (2012). Classification of *Camellia* (Theaceae) species using leaf architecture variations and Pattern recognition technique. *PLOS One* 7(1): e29704. doi: 10.1371/journalpone.0029704.

Metcalfe, C.R. and L. Chalk (1979). *Anatomy of the Dicotyledons (Second Ed.). Vol. I. Systematics, Anatomy of Leaf and Stem with Brief history of the Subject*. Oxford, 176 pp.

Nassar, M.I., E.A. Abutabl, R.F. Ahmed., E.A. El-Khrisy, K.M. Ibrahim and A.A. Saleem (2010). Secondary metabolites and bioactivities of *Myrtus communis* L. *Pharmacognosy Res.,* 2(6): 325-329.

Parkhurst, D.F. (1978). Adaptive significance of stomata occurrence on one or both surfaces of leaves. *J. Ecology* 66: 367-383.

Persaud, N., M. Gandah, M. Ouatarra and N. Mokete (1993). Estimating leaf area of Pearl Millet from linear measurements. *Agron. J*., 85: 10-12.

Prabhakar, M. (2004). Structure, delimitation, nomenclature and classification of stomata. *Acta Botanica Sinica*, 46 (2): 242-252.

Retamales, H.A., R. Scherson and T. Scharaschkin (2014). Foliar micro-morphology and anatomy of *Ugni molinae* Turez. (Myrtaceae), with particular reference to schizogenesis secretory cavities. *Revista Chilena de Historia Natural*.87: 27 (DOI: 10.1186/s40693-014-0027-x)

Robbins, S.N. and D.M. Pharr (1987). Leaf area prediction models for cucumber from linear measurements. *Hort Science*, 22: 1264-1266.

Rotondi, A., F. Rossi, C. Asunis and C. Cessaraccio (2003). Leaf xeromorphic adaptations of some plants of a coastal Mediterranean machia ecosystem. *J. Mediterranean Ecology* 4(3): 25-35.

Sara, S., M. Ibrar, Barkatullah, N. Muhammad and M. Ehsan (2013). Analgesic and gastro-intestinal motility profile of essential from *Myrtus communis* leaves. *Phyto-Pharmacology* 4(1): 81-86.

Shaukat, S.S. and D. Khan (1979). A comparative study of the statistical behaviour of diversity and equitability indices with reference to Desert vegetation. *Pak. J. Bot.,* 22(2): 155-165.

Sumbul, S., M.A. Ahmad, M. Asif and M. Akhtar (2011). *Myrtus communis* Linn. –A Review. *Ind. J. Nat. Products and Resources* 24 (4); 395-402.

Trewartha, S.T. (1954). *An Introduction to Climate*. McGraw Hill, Inc.

Uzun, S. and H. Çelik (1999). Leaf area prediction models (Uzcelik-I) for different horticultural plants. *Tr. J. Agric. & Forestry*, 23: 645–650.

Wang, Xiu-Wei, Mao Zi-Jun, Choi, Kyung and Park, Kwang-Woo (2006). Significance of the leaf epidermis fingerprint for taxonomy of Genus *Rhododendron*. *J. Forest. Res*., 17(3): 171-176.

Williams, L. III and T.F. Martinson (2003). Nondestructive leaf area of 'Niagara' and DeChaunac' grapevines. *Scientia Horticulturae*, 98(4): 493 – 498.

Yadav, R.K.P., A.M. Basabalidas and D. Vokou (2004). Leaf structural features of Mediterranean perennial species: Plasticity and Life form specificity. *J. Biol. Res.* 2:21-34.

Zar, J.H. (2010). *Biostatistical Analysis*. 5th Ed. Prentice-Hall, Englewood Cliffs. New Jersey, USA.

Zubenok, L.I. (1977). Annual Potential Evapotranspiration Map Sheet No 18. In: *Atlas of World Water Balance* (M.I. Budyko, Ed.) UNESCO Press, Paris.

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